6. DISCUSSION

OKRA GUM

Isolation

Three different methods (Method A, Method B and Method C) were used for the isolation of OG to obtain better yield from the okra pods. Method C gave better percentage of yield (37.5% wt/wt) as compared to Method B (15% wt/wt) and Method A (3.75% wt/wt). Method B requires more quantity of ethanol for washing. This method also requires centrifuge for the process of centrifugation for separation of gum from dispersion that was difficult and tedious. Method A was time consuming and requires more quantity (1 to 1.5L liter) of solvent (acetone) for the precipitation of OG where separation of gum from the parent solution was very difficult. Further, fungal growth was observed on OG after storage of a single day which produced foul smell. Thus it requires much more facilities for storage. Method C gave greenish black light weighted flakes of OG which were size reduced to get a fine free flowing powder. Thus in comparison to all employed methods for isolation of OG, Method C was relatively economical, gave better percentage of yield with required characteristics of products (fluffy and non-sticky). Due to these reasons Method C was selected for further study.

Phytochemical examination

Various phytochemical tests were carried out on obtained okra gum powder. On treatment with ruthenium red, it showed red color confirming the obtained product as mucilage. A violet ring was formed at the junction of two liquids on reaction with Molisch’s reagent indicating the presence of carbohydrates. Gum could not reduce Fehling’s solution, so the sugars present were non reducing sugars. It reduces Fehling’s solution after hydrolysis for 1h with concentrated sulfuric acid under reflux. Gum on treating with ninhydrin reagent did not gave
purple coloration indicating the absence of amino acids. The results of phytochemical screening of okra gum are summarized in Table 4.1.

**Physicochemical properties**

The extracted okra gum was evaluated for various physicochemical properties and their results are shown in Table 4.2. The extracted okra gum was slightly soluble in water as one gram of gum required about 500mL of water to dissolve it and a dispersion of it yielded a dark green solution. The swelling characteristic of OG was studied in different medium like 0.1N HCl pH 1.2, PBS pH 7.4, PBS pH 6.8 and distilled water (DW). The swelling indices were almost identical and their values were ranged from 2.15 to 2.38. It was also found that swelling was irrespective of pH of swelling medium and thus it may be utilized for the formulation of oral dosage forms which encountered variation in pH during their GI transit. The moisture content of OG was low (0.89 ± 0.01%), suggesting its suitability in the formulation containing moisture sensitive drugs. The total ash and acid insoluble ash values of OG were 1.8% wt/wt and 0.6% wt/wt, respectively. These Ash values reflected the level of adulteration in the gum. The low values of total ash and acid insoluble ash obtained in this study showed low levels of contamination during gathering and handling of crude okra. Angle of repose (θ) is a traditional characterization method for pharmaceutical powder to check the flow property. There is a correlation between the flow property and angle of repose: the better the flow property, the smaller the angle of repose. A value of <30° shows 'excellent' flow whereas >56° shows 'very poor' flow. The intermediate scale showed as 'good' (θ between 31-35°), 'fair' (θ between 36-40°), 'passable which may hang up' (θ between 41-45°), and 'poor which must be agitated or vibrated' (θ between 46-55°). Based on these data, the flow of extracted OG was rated as 'good' because θ was found to be 32°. A 1% wt/vol suspension of OG in water gave a pH of 6.8. Thus near neutral pH of OG suspension indicated that it might be useful in the formulations containing acidic, basic and neutral drugs. Lower bulk density (0.72 ± 0.021g/cm³) and higher tapped density (0.84 ±
0.031g/cm³) for OG powder favors packing behavior. These parameters may give good mass and content uniformity of dosage forms formulated from such gum powder. Bulk density and tapped density can also provide information on the flow ability of the powders. By using bulk density and tapped density, the Carr’s index (CI) and Hausner’s ratio (HR) were calculated. Lower CI or lower HR of a material showed better flow properties than higher ones. A CI of <10% or HR of <1.11 are considered to 'excellent' flow where as CI>38% or HR>1.60 are considered to be 'very very poor' flow. The intermediate scales showed ‘good flow’ (CI between 11-15% or HR between 1.12-1.18), ‘Fair flow’ (CI between 16-20% or HR between 1.19-1.25), ‘passable flow’ (CI between 21-25% or HR between 1.26-1.34), 'poor flow’ (CI between 26-31% or HR between 1.35-1.45) and 'very poor flow’ (CI between 32-37% or HR between 1.46-1.59). Based on these data, flow of OG was rated as 'good' because CI and HR were found to be 14.3 ± 0.014% and 1.16 ± 0.014, respectively.

Instrumental analysis

Thermo gravimetric analysis (TGA)

The thermogravimetric analysis (TGA) was used to determine the weight loss of the material on heating. Transitions involving mass changes are detected by TGA as a function of temperature and time. TG curve for OG is shown in Figure 2.1. Obtained TG curve revealed that the weight loss of OG was due to loss of water around 50-200°C in stages. The curve shows that the gum did not decomposed before 200°C. Several previous studies (De Rosa et al., 2010; Threepopnatkul et al., 2009) revealed that the loss in weight during this stage was due to the release of absorbed moisture or vaporization of the water from the gum. It has been reported that water is formed by intra- and intermolecular condensation of polymeric hydroxyl groups which are the main product of decomposition at temperature below 300°C. The gum underwent 13.46% weight loss at 200°C. The second stage of weight loss occurred at 200°C -
580°C, with the peak of this transition at 225°C. This weight loss indicated the decomposition of cellulose. It implies that OG has excellent thermal stability.

Differential scanning calorimetry (DSC)

DSC was used to measure the occurrence of exothermal or endothermal changes with increase in temperature. DSC, because of its sensitivity and accuracy, has been extensively used to study the phase transitions of polymers. The thermogram for OG is shown in Figure 2.2. This thermogram showed that the gum has both amorphous and crystalline portions. Glass transition temperature (Tg) occurred at 79.47°C. The continuous (broad) endothermic transition that followed the glass transition is indicative of crystallite melting occurring over the glass transition range. The Tg was also observed to be high, indicating a high degree of crystallinity of the gum. This high degree of crystallinity provides useful structural stability of gum and the granules prepared from such gum powder may be more resistant to heat due to this. It has also been reported that materials of low Tg have low crystallinity. The knowledge of Tg is essential in production processes and storage as Tg is affected by moisture and other additives, facilitating conversion to the rubbery state and hence facilitating crystallization through molecular rearrangement. The gum was also observed to have low enthalpy; this is attributed to the presence of regular small and oval granules.

Fourier transform-infra red (FT-IR)

FT-IR spectrum of OG (Figure 2.3) reported principally as a fingerprint for any future identification, since the absorption bands for such complex compounds are usually broad and diffuse. The band at 1626cm\(^{-1}\) was assigned to the O-H bending of water. There are weak peak in the 1736cm\(^{-1}\) area that indicates presence of carbonyl groups. The sharp band at 2928cm\(^{-1}\) is characteristic of methyl C-H stretching associated with aromatic rings. The broad band at 3427cm\(^{-1}\) is due to the hydrogen-bonding that contributes to the complex
vibrational stretches associated with free inter and intra-molecular bound hydroxyl groups which make up the gross structure of carbohydrates.

X-ray powder diffraction (XRPD)

The X-ray diffractogram of okra gum is shown in Figure 2.4. The Bragg reflection angle, $2\theta$, along with the interplanar spacing, $d$, and the relative intensity of the peaks were reported. The sample shows peaks at approximately 14.9284° and 38.3015°, $2\theta$. However, other peaks were very weak and unresolved. The result of XPRD shows that OG had both amorphous and crystalline portions.

Particle size analysis of OG

Particle size analysis revealed that gum has uniform size distribution (Figure 2.5). Various parameters like specific surface area, surface mean diameter, volume mean diameter and specific volume area were found to be 355.42cm$^2$/g, 62.29µm, 219.95µm and 0.10m$^2$/cm$^3$, respectively.

Elemental analysis

The low level of nitrogen is suggestive of amino acid (peptide) crosslink in the sample. The ratio of carbon to hydrogen is just over 6:1 indicating a good number of unsaturation due to aromatic rings and/or polysaccharide composition.

Microstructure study

A scanning electron micrograph of okra gum is shown in Figure 2.6. The micrograph is indicative of an amorphous material. The particles were mostly seen as aggregates of irregular shapes and dimensions which were fibrous in nature. The shape and structure or surface topography of the polysaccharide gum may be affected by the method of extraction and purification or preparation of the product (Qian et al., 2009).
Functional property

pH change study

Biodegradation study of natural polysaccharide by colonic bacteria may help to predict the possible clinical application of the polysaccharide substances. This study was based on the principle that when a natural polysaccharide degraded by bacteria an organic acid forms which decrease the pH value of dissolution medium containing rat caecal content. It was found that the pH of dissolution medium rapidly decreases by the addition of OG which may be due to the degradation of OG by colonic bacteria (bacterial enzymes) in the caecal contents to generate an organic acid thereby reducing the pH (Figure 2.7). These changes were almost certainly caused by bacterial action. The pH change was found to be drastic from the initial pH 6.82 to 4.12 pH units in the test compare to very little change in the pH of the control viz. pH 6.8 to 6.5 pH units. The smaller reduction in pH in the control could probably be explained by bacterial fermentation of substrate already present in the rat caecal.
THEOPHYLLINE

Identification of drug

Identification of Theophylline was performed by melting point study and IR spectroscopy study. Melting point of Theophylline was found in accordance with the standard melting point of Theophylline as per British Pharmacopoeia. Data of IR vibrations and its frequency are depicted in Table 4.3 and they were in accordance with standard IR spectrum of Theophylline (Figure 2.8).

Preformulation study by Differential Scanning Calorimetry

The DSC thermogram (Figure 2.9) of Theophylline did not show any major change in the endothermic peak with tablet formulation (Figure 2.10). However, slight shifts at the peak location of Theophylline towards lower temperature and decrease in the peak intensity were demonstrated. The formulation also showed changes in the heat of fusion (ΔH) values. So, this all finding suggested that drug, polymer and excipients does not reacted adversely in the formation of tablets.

Spectrophotometric analysis of Theophylline

Determination of λ_{\text{max}} of Theophylline in PBS pH 6.8

The UV visible spectrum of Theophylline in PBS pH 6.8 is shown in Figure 2.11 (spectrum) and the λ_{\text{max}} was found to be 274nm.

Standard curve of Theophylline in different media

The absorbance values of different concentration of Theophylline (2.5-20µg/mL) in different media viz. 0.1N HCl pH 1.2, phosphate buffer saline pH 7.4 and phosphate buffer saline pH 6.8 are depicted in Table 4.4. The values of R² were found to be 0.999 (Figure 2.12), 0.999 (Figure 2.13) and 0.998 (Figure 2.14) for 0.1N HCl pH 1.2, phosphate buffer saline pH 7.4 and phosphate buffer saline pH 6.8, respectively which indicate the accuracy of results.
A comparative study of physical properties of matrix tablets

This study was carried out to check the tableting properties of OG in comparison to other extensively used polymers viz. Guar gum (GG), Xanthan gum (XG), Locust bean gum (LBG), chitosan (CHI) and Hydroxy propyl methyl cellulose (HPMC). The formulations (F1-F6) were prepared as shown in Table 3.1 (F1-F6).

Granulation is the key process in the production of many dosage forms. Physical properties of granules such as specific surface area, shape, hardness, surface characteristics and size can significantly affect the rate of dissolution of drug contained in a formulation.

Thus, the granules of the different formulations (F1-F6) were evaluated for angle of repose, bulk density, tapped density, Carr’s compressibility index (CI) and Hausner’s ratio (HR). Results of granulation properties are shown in Table 4.5. No significant differences in apparent densities were observed for granules of different batches. Lower bulk density (0.224 ± 0.012 g/cm³ to 0.355 ± 0.021 g/cm³) and higher tapped density (0.345 ± 0.013 g/cm³ to 0.542 ± 0.013 g/cm³) were found for all the granules which favors packing behavior, resulting in good mass and content uniformity of dosage forms formulated from such granules.

The angle of repose (θ) is a traditional characterization method for pharmaceutical powder to check flow property. There is a correlation between the flow property and angle of repose: smaller angle of repose (θ <30°) gives better flow property. It is well known that angle of repose decreases with an increase of powder flowability and so it was used as a parameter of the powder flowability. The height of the granules (h) forming the cone and the radius (r) of the base were measured. The angle of repose was calculated using an equation [4].

Flowability is indicated based on the angle of repose (θ). A value θ<30° indicates ‘excellent’ flow property whereas θ>56° indicates ‘very poor’ flow property. The intermediate scale indicates ‘good’ (θ between 31°–35°), ‘fair’ (θ
Discussion

between 36°–40°), ‘passable which may hang up’ (θ between 41°–45°) and ‘poor which must be agitated or vibrated’ (θ between 46°–55°). Based on this data, the flow of prepared granules was rated as ‘poor, must agitate or vibrate’ for the formulations F1 (51.78 ± 1.7°), F2 (47.70 ± 0.85°), F3 (48.61 ± 2.7°) and F6 (47.64 ± 1.2°) whereas F4 (63.40 ± 1.2°) and F5 (56.74 ± 0.4°) were rated as ‘very poor flow’.

Bulk density and tapped density can also provide information on the flow ability of the powders. By using bulk density and tapped density, CI and HR were calculated. The lower the CI is the better the flowability of the powder. Granules with lower CI relates to better powder bed stability and flow properties of the granules. Lower CI or lower HR of a material indicates better flow property than higher ones (Carr RL, 1965; Hausner HH, 1967). A CI of <10% or HR of <1.11 is considered ‘excellent’ flow where as CI>38% or HR>1.60 is considered ‘very very poor’ flow. There are intermediate scales for CI between 11–15% or HR between 1.12–1.18 is considered ‘good’ flow, CI between 16–20% or HR between 1.19–1.25 is considered ‘fair’ flow, CI between 21–25% or HR between 1.26–1.34 is considered passable flow, CI between 26–31% or HR between 1.35–1.45 is considered ‘poor’ flow, and CI between 32–37% or HR between 1.46–1.59 is considered ‘very poor’ flow. Based on the results obtained, flow of granules of formulation F1 was rated as ‘fair’, compared to flow of granules of the formulations F2 and F3 which were rated as ‘very poor’ and flow of granules of the formulations F4, F5 and F6 were considered to be ‘very very poor’ in terms of their flow property. This discrepancy might be due to very qualitative nature of the scale of measurements and ratings for flow properties based on these compendial methods.

Physical evaluation of matrix tablets of Theophylline

The tablets of different formulations were subjected to various evaluation tests, such as weight variation, friability, hardness and drug content according to procedure specified in methodology section. The results of weight variation
(≤2.43%), friability (≤0.78%), hardness (4.0 kg/cm² to 4.5kg/cm²) and drug content (97.56% to 99.34%) are depicted in Table 4.6. Thus, all the physical properties of these prepared tablet formulations (F1-F6) were satisfactory.

Swelling and erosion study

The aqueous medium on contact with hydrophilic polymer matrix gradually begins to hydrate from the periphery towards the centre, forming a gelatinous swollen mass, which controls the diffusion of drug molecules through the polymeric material into aqueous medium. The hydrated gel layer thickness determines the diffusional path length of the drug. The swelling behavior indicates the rate at which this formulation absorbs water from dissolution media and swells. Thus prepared formulations (F1-F6) were comparatively evaluated for their swelling and erosion characteristics for 6h. These formulations exhibited varied degree of swelling as shown in Table 4.7. It was observed that formulation (F5) containing chitosan starts disintegration immediately when contacted with swelling medium and disintegrated within 3min owing to protonation of chitosan and then erosion of tablets (Thawatchai et al., 2008). Visual observation of remaining formulations showed that the matrices appeared swollen almost from the beginning and a viscous gel mass was created when they came into contact with the swelling medium. Based on the degree of swelling, the formulations arranged in descending order of their degree of swelling viz. F4>F2>F3>F6>F1 (Figure 2.15). Figure 2.16 illustrates the photographic images of swelling study for the prepared formulations (F1-F4 & F6) after 1h. On the other hand matrix erosion study measured the weight loss from matrix tablets immersed in dissolution media as a function of time was determined. The weight loss of the tablets was study upto 6h, which illustrates matrix erosion profiles of the formulation and indicates their inverse relationship with water uptake (Table 4.8). It follows that the hydrated formulation network maintains its tight integrity with drug release by erosion and dissolution of the drug accounting for most of the weight loss during the remainder of the experimental period. As per the erosion study the formulations
were arranged in descending order starting from higher erosion rate to lower one viz. F1>F3>F2>F6>F4 (Figure 2.17).

It was found that a rapid erosion of the hydrated layer, releases most of the drug content within 5h and higher degree of swelling with lower rate of erosion showed subsequent sustained release of drug. These may attributes to polymer viscosities because the formulation containing GG (F2) and LBG (F4) with viscosity of 240cp and 250cp, respectively which showed higher degree of swelling and lower rates of erosion compare to formulation containing XG (F3) and HPMC (F1) with viscosity of 150cp and 157cp, respectively which showed lower degree of swelling and higher rates of erosion. The higher viscosity will have higher and faster water absorption capacities and tend to swell more rapidly. The hydration and the swelling rate of these matrix tablets relates to the hydroxypropyl substitutes percentage on the polymer and the concentration of this polymer per tablet. HPMC contains the highest amount of these groups and produces strongly viscous gel that increases integrity and resistance of the matrix to erosion. The extent of polymer swelling and the hydration of the microstructure formed within the gel layer vary in accordance with polymer interaction with the hydrating media. The gums are more hydrophilic and therefore showed greater water uptake and erosion than HPMC. Exceptionally, formulation F6 (OG) showed lower degree of swelling as well as lower degree of erosion. This may be due to its pH value which is about 6.8 and is soluble at near neutral pH. Due to its low solubility, it was difficult to hydrate in 0.1N HCl pH 1.2 that reduces its water uptake and swelling capacity.

Dissolution study

A significant difference in release pattern was observed among the formulations (F1-F4 & F6) as shown in the Table 4.9. An initial burst of 10.05 ± 2.1%, 30.17 ± 2.1%, 24.15 ± 2.2%, 30.68 ± 0.9% and 13.10 ± 1.1% were found for the formulations F1, F2, F3, F4 and F6, respectively after 1h. The burst phenomenon may be due to surface erosion or initial disaggregation of the matrix tablet prior to gel layer formation around the tablet core (Kuksal et
At the end of 5h of dissolution period it was found that 46.78 ± 0.9%, 60.54 ± 1.4%, 61.42 ± 1.1%, 72.82 ± 1.6% and 51.42 ± 2.4% of drug release for the formulations F1, F2, F3, F4 and F6, respectively (Figure 2.18). This variation in release pattern may be due to the different properties of the polymers especially the viscosity and hydration. Drug release was generally linear for most of the formulations, especially formulation containing LBG (F4). Such linear release from hydrophilic matrices may be due to synchronization between swelling and erosion of the polymer in maintaining a constant gel layer.

LBG is a nonionic polysaccharide and the hydration process is independent of pH but will degrade at pH extremes at higher temperatures. LBG is less soluble and has lower viscosity than guar gum as it has fewer galactose branch points which makes release faster. Guar gum hydrates rapidly in cold water to give highly viscous pseudoplastic solution of high viscosity as compared to other hydrocolloids and greater than that of LBG. During the dissolution study, all the formulations swelled and the outer layer of most of the tablets appeared to be hydrated after being placed in dissolution medium, with a progressive increase in the size of this hydrated layer, especially evident for formulation containing XG (F3), followed by a gradual loss of integrity due to hydrodynamic stress induced by the dissolution apparatus. XG is mainly considered to be non-gelling and used for the control of viscosity due to the tenuous associations endowing it with weak-gel shear-thinning properties. The sustained release of drug from formulation containing HPMC (F1) may be due to the hydroxypropyl substitutes percentage on the polymer. HPMC contains higher amount of these groups and produces strongly viscose gel that increases integrity and resistance of the matrix to erosion which might be responsible for slow drug release (Moin and Shivakumar, 2010; Salsa et al., 1997).

The tableting properties of extracted okra gum were found satisfactory and were comparable with tableting properties of extensively used semi synthetic HPMC.
Preliminary intact study of matrix tablets containing 300mg of Theophylline

The successful delivery of drug to colon requires the protection of drug from being released in the stomach and small intestine. In present section, matrix tablets were prepared using okra gum alone and in combination with other gums (guar gum, locust bean gum and chitosan) and preliminary intact study were carried out in the physiological environment of stomach and small intestine to check the tablet intactness.

Preparation of matrix tablets by direct compression method

Eighteen batches of tablets IS1 to IS4 (Table 3.2) and IS5 to IS18 (Table 3.3) were prepared using direct compression method containing okra gum alone and in combinations with above said gums. These prepared tablets were evaluated for weight variation, hardness and preliminary intact study. The results of evaluation tests are depicted in Table 4.10 (IS1-IS4), Table 4.11 (IS5-IS10) and Table 4.12 (IS11-IS18). It was observed that matrix tablets had relatively poor hardness (≤2.5kg/cm²) at maximum compression force. Prepared tablets were subjected to preliminary intact study for 5h, initially 2h in simulated gastric fluid, SGF, (0.1N HCl, pH 1.2) followed by 3h in simulated intestinal fluid, SIF, (Phosphate buffer saline, pH 7.4). It was found that tablet formulations containing chitosan as one of the matrix components (IS4, IS7, IS10, IS13 and IS16) showed immediate disintegration and disintegrates within 10min. Thus chitosan was not able to prevent the drug release in upper GIT.

The formulations containing LBG (IS2, IS5, IS8, IS11, IS14 and IS17) and guar gum (IS3, IS6, IS9, IS12, IS15 and IS18) were disintegrated completely within 30min. It was found that prepared tablets of said batches were not able to prevent the release of drug in the upper environment of GIT. This phenomenon occurred presumably as a result of the lower crushing strength (≤2.5kg/cm²) of tablets due to which tablets disintegrated rapidly. It was
observed that tablet formulations containing OG and GG undergo swelling and erosion simultaneously followed by complete erosion as compared to tablet formulations containing OG and LBG which undergo erosion layer by layer. This may be due to rapid hydrating property of GG because it consists of higher galactose points as compared to LBG.

Preparation of matrix tablets by wet granulation method

Further study was carried out using wet granulation method and eighteen batches of tablets IS19 to IS22 (Table 3.4) and IS23 to IS33 (Table 3.5) were prepared. These prepared tablets were evaluated for weight variation, hardness and preliminary intact study. The results of evaluation tests are depicted in Table 4.13 and Table 4.14. The matrix tablets prepared by wet granulation method were found to have improved crushing strength (3.5-4.6kg/cm²). Prepared tablets were subjected to preliminary intact study for 5h, initially 2h in simulated gastric fluid, SGF, (0.1N HCl, pH 1.2) followed by 3h in simulated intestinal fluid, SIF, (Phosphate buffer saline, pH 7.4). The tablet formulations containing blends of OG and chitosan (IS22, IS25, IS28 and IS31) were disintegrated completely within 2h. This may be due to protonation of chitosan with subsequent erosion as well as to high solubility of cationic chitosan in acidic media leading to fast disintegration within a short period of time. The tablet formulations containing combinations of OG with LBG (IS20, IS23 and IS26) and OG with GG (IS21, IS24 and IS27) showed immediate swelling followed by complete disintegration in SIF. Tablet formulations containing higher concentration of OG with LBG (IS29 and IS32) and higher concentration of OG with GG (IS30 and IS33) showed slow erosion without swelling.

During preliminary intact study of tablet formulations (IS19-IS33) two samples each of 1mL were taken at the end of 2h and 5h of study, suitably diluted and analyzed for drug content at respective λ\text{max} using UV visible spectrophotometer. The results are shown in Table 4.15. The tablet
formulations containing blends of OG and chitosan (IS22, IS25, IS28 and IS31) gave complete drug release in SGF due to protonation of chitosan with subsequent erosion as well as to high solubility of cationic chitosan in acidic media, (Rege, 1999; Sabnis, 1996; Upadrashta and Katikaneni, 1992) leading to fast disintegration within a short period of time. The tablet formulations containing combinations of OG with LBG (IS20, IS23 and IS26) and OG with GG (IS21, IS24 and IS27) were not able to prevent in upper GIT with maximum cumulative drug release of about 55%. This may be due to the fact that when the aqueous medium on contact with hydrophilic polymer matrix it gradually begins to hydrate from the periphery towards the centre, forming a gelatinous swollen mass, followed by drug diffusion through the polymeric material into aqueous medium. It was found that as the amount of LBG and GG in the formulations was increased, there was a greater degree of gum hydration with simultaneous swelling with a progressive increase in the size of this hydrated layer followed by a gradual loss of integrity, resulting from the hydrodynamic stress induced by the dissolution apparatus responsible for higher drug release. Tablet formulations containing higher concentration of OG with LBG (IS29 and IS32) and higher concentration of OG with GG (IS30 and IS33) found to restricted the drug release in upper GIT with ≤10.60% of drug release. This phenomenon occurred presumably as a result of the high crushing strength of tablets and because of solubility property of okra gum. The OG dispersion has a pH of about 6.8 and OG is soluble around neutral pH. Due to its low solubility, it was difficult to hydrate in 0.1N HCl and thus OG prevents the drug release in upper GIT.

Tablet formulations (IS34-IS36) were prepared using increasing concentration of OG by wet granulation method as shown in Table 3.6. These prepared tablets were evaluated for weight variation, hardness and preliminary intact study. The results of evaluation are depicted in Table 4.16. It was observed that matrix tablets had good hardness (3.7 ± 0.54kg/cm² to 4.4 ± 0.22kg/cm²) and weight variation was ≤3.4%. Prepared tablets were subjected to preliminary
intact study for 5h, initially 2h in simulated gastric fluid, SGF, (0.1N HCl, pH 1.2) followed by 3h in simulated intestinal fluid, SIF, (Phosphate buffer saline, pH 7.4). These prepared formulations were found remained intact in the physiological environment of stomach (SGF) and small intestine (SIF) for 5h without swelling and erosion.

Tablet formulations (IS37-IS39) containing 200mg Theophylline were prepared using increasing concentration of OG by wet granulation method as shown in Table 3.7. The rationale of using 200mg Theophylline was to accommodate maximum amount of release rate controlling polymers in the formulations so as to restrict further drug release in upper GIT. These prepared tablets were evaluated for weight variation, hardness and preliminary intact study. The results of evaluation tests are depicted in Table 4.17. It was observed that matrix tablets had good hardness (4.2 ± 0.42kg/cm² to 4.4 ± 0.35kg/cm²) and weight variation within the limit (≤3.2%). Prepared tablets were subjected to preliminary intact study for 5h, initially 2h in simulated gastric fluid, SGF, (0.1N HCl, pH 1.2) followed by 3h in simulated intestinal fluid, SIF, (Phosphate buffer saline, pH 7.4). These prepared formulations were found remained intact in the physiological environment of stomach (SGF) and small intestine (SIF) for 5h without swelling and erosion.

During preliminary intact study of tablet formulations (IS34-IS39) two samples each of 1mL were taken at the end of 2h and 5h of study, suitably diluted and analyzed for drug content at respective λ_max using UV visible spectrophotometer. The results are shown in Table 4.18. The cumulative drug release was found to be decreased with formulations containing higher amount of OG. It was found that formulations (IS34-IS36) containing 300mg of drug showed decreased in values of Rel_5h from 21.11% (IS34) to 10.11% (IS37). Formulations (IS37-IS39) containing 200mg of drug showed further decreased in values of Rel_5h from 4.56% (IS36) and 2.21% (IS39). This is due to the fact that the lower concentration of drug (200mg) in the formulation permits higher concentration of OG that increases density of gum matrix resulted in an
increased diffusional path length and higher crushing strength of tablets. Moreover, OG dispersion has a pH of about 6.8 and OG is soluble around neutral pH. Due to its low solubility, it was difficult to hydrate in 0.1N HCl and thus OG prevents the drug release in upper GIT.

Based on the preliminary intact study it was found that tablets prepared using direct compression method leads to faster disintegration in the dissolution medium because of poor binding property. Hence, further batches of tablets containing 200mg of Theophylline were prepared by using wet granulation methods.

**Preparation of matrix tablets by wet granulation method for colon specific drug delivery**

Preparation of matrix tablets by PVPK30

To prepare colon specific drug delivery system using natural gums it is the prime requirement of formulation that it should not release the drug in upper GIT i.e. stomach and small intestine and when it reaches to colon it should abruptly release the drug. Tablet formulations (OG1-OG3) were prepared using drug and OG in the ratio of 1:0.5, 1:1 and 1:1.5 by wet granulation method as shown in Table 3.8. A 3% PVPK30 was used as binder and granulation was done using 22/44# sieves. These formulations were evaluated for weight variation, friability, hardness, drug content and Rel\textsubscript{5h} as procedure mentioned in methodology section 4.2.5. Results of weight variation (≤3.12%), friability (≤0.64%), hardness (3.1 ± 0.67kg/cm\textsuperscript{2} to 3.6 ± 0.64kg/cm\textsuperscript{2}), drug content (98.12% to 101.24%) and Rel\textsubscript{5h} (64.35% to 92.64%) for prepared matrix tablets are shown in Table 4.19. Tablet formulations (OG4-OG6) were prepared using drug and OG in the ratio of 1:0.5, 1:1 and 1:1.5 by wet granulation method as shown in Table 3.9. A 3% PVPK30 was used as binder and granulation was done using 60# sieves so as to improve crushing strength. Tablets prepared from smaller granules exhibited greater compactibility compared to tablets made from larger granules and hence a higher tensile strength (Eichie and
Kudehinbu, 2009). These formulations were evaluated for weight variation, friability, hardness, drug content and Rel$_{5h}$ as procedure mentioned in methodology section. Results of weight variation ($\leq 1.26\%$), friability ($\leq 0.64\%$), hardness ($3.1 \pm 0.67$kg/cm$^2$ to $3.6 \pm 0.64$kg/cm$^2$), drug content ($96.35\%$ to $98.67\%$) and Rel$_{5h}$ ($60.31\%$ to $88.64\%$) for prepared matrix tablets are shown in Table 4.20.

Further study was carried out where granulation was done by using 60# sieve as it was found that decreasing particle size of granules increases the crushing strength and might be responsible for controlled drug release in upper GIT.

Tablet formulations OG7 to OG10 (Table 3.10) and OG11 to OG13 (Table 3.11) were prepared with $3.5\%$ and $3.75\%$ PVPK30, respectively using increasing concentrations of OG. These formulations were evaluated for weight variation, friability, hardness, drug content and Rel$_{5h}$ as procedure mentioned in methodology section 4.2.5. Results of weight variation ($\leq 2.23\%$), friability ($\leq 0.89\%$), hardness ($4.25 \pm 0.34$kg/cm$^2$ to $5.54 \pm 0.30$kg/cm$^2$), drug content ($97.78\%$ to $102.10\%$) and Rel$_{5h}$ ($27.74\%$ to $84.11\%$) for prepared matrix tablets (OG7-OG10) are shown in Table 4.21. It was found that tablet formulation containing higher concentration of OG (400mg) restricted drug release ($27.74\%$) in upper GIT satisfactorily. This is due to higher gum concentration with good binding property produced by PVPK30 ($3.5\%$) in the formulation. Results of weight variation ($\leq 3.35\%$), friability ($\leq 0.89\%$), hardness ($4.25 \pm 0.11$% to $4.50 \pm 0.30$kg/cm$^2$), drug content ($96.45\%$ to $197.70\%$) and Rel$_{5h}$ ($55.84\%$ to $89.64\%$) for prepared matrix tablets (OG11-OG13) are shown in Table 4.22. Obtained cohesive mass after addition of binder during preparation of tablet formulations (OG11-OG13) containing higher concentration of PVPK30 ($3.75\%$) was found to be very sticky and was difficult to be sieved. This might leads to non uniformity of content and results in fast release of drug from the said tablet formulations.

The drug delivery systems targeted to the colon should not only protect the drug from being released in the physiological environment of stomach and
small intestine, but also release the drug in colon after enzymatic degradation by colonic bacteria. Hence, drug release study was carried out with PBS pH 6.8 in presence or absence of rat caecal content. Cumulative percentage drug release from the formulations OG7-OG10 and OG11-OG13 in presence and absence of rat caecal content are shown in Table 4.23 and Table 4.24, respectively. Comparison of dissolution profiles for the formulations OG7-OG10 and OG11-OG13 are shown in Figure 2.19 and Figure 2.21, respectively. Photographs of matrix tablets (OG8, OG9 and OG10) during their dissolution are shown in Figure 2.20. Dissolution study in presence or absence of rat caecal content revealed that drug release in presence of rat caecal content was increased to a great extent as compared to drug release in absence of rat caecal content (control) for the above said formulations except (OG7 and OG11). Moreover, similarity factor ($f_2$) was used to compare dissolution profiles for the formulations (OG7-OG13) in presence and absence of rat caecal content. The $f_2$ values for comparison of dissolution data of tablet formulations (OG7-OG13) were found to be less than 50 indicating that drug release profiles of the formulations in presence and absence of rat caecal content are dissimilar except for the formulations OG7 and OG11 whose $f_2$ value were 84.92 and 87.34, respectively (similar dissolution profiles). So it was concluded that in presence of rat caecal contents the drug release from the formulations was increased significantly. It may be due to polysaccharidases which are present in the caecal matter that metabolizes the gum.

Further tablet formulations OG14 to OG23 (Table 3.12) were prepared to check the synergistic effect of okra gum and other polymers like HPMC/pectin/amylose/ethyl cellulose. These formulations were evaluated for weight variation, friability, hardness, drug content and Rel$_{5h}$ as procedure mentioned in methodology section 4.2.5. Results of weight variation (≤2.1%), friability (≤0.89%), hardness (3.25 ± 0.78kg/cm$^2$ to 5.3 ± 0.67kg/cm$^2$), drug content (96.15% to 101.4%) and Rel$_{5h}$ (29.14% to 101.20%) for these formulations are shown in Table 4.25. Tablet formulation (OG16) containing
OG (300mg) and amylose (100mg) showed fast erosion. This may be due to higher swelling power of amylose which could not produce stable gel layer and eroded completely. Formulations (OG15, OG19 and OG22) containing OG (300mg, 300mg and 400mg, respectively) and pectin (100mg, 200mg and 75.5mg, respectively) showed somewhat less erosion. This may be due to fact that pectin has a pH of 3-4, thus in acidic medium it can be converted to pectinic acid which has lower swelling capacity compare to pectin (Sang et al., 2006). Formulations (OG17, OG18 and OG21) containing OG (300mg, 300mg and 400mg, respectively) and EC (100mg, 200mg and 75.5mg, respectively) showed less erosion but some of the tablets were found to be broken down in to two halves during dissolution. Formulations (OG14, OG20 and OG23) containing OG (300mg, 300mg and 400mg, respectively) and HPMC (100mg, 200mg and 75.5mg, respectively) showed very low erosion. This may be due to higher viscosity and stability of gel layer formed by HPMC compare to other polymers. Among prepared tablets, formulation (OG23) containing higher amount of OG (400mg) and lower amount of HPMC (75.5mg) showed restricted drug release in upper GIT (Rel_{5h}=29.14%). This may be due to synergistic effect of HPMC (high viscosity and stable gel forming property) and OG (lower solubility in 0.1N HCl, pH 1.2). The drug delivery systems targeted to the colon should not only protect the drug from being released in the physiological environment of stomach and small intestine, but also release the drug in colon after enzymatic degradation by colonic bacteria. Hence, drug release study was carried out with PBS pH 6.8 in presence or absence of rat caecal content. Cumulative percentage drug release for the prepared tablets (OG21-OG23) is given in Table 4.26. Comparative dissolution profiles of prepared tablets (OG21-OG23) are shown in Figure 2.22. Moreover, similarity factor ($f_2$) was used to compare dissolution profiles for the formulations (OG24-OG29) in presence and absence of rat caecal content. The $f_2$ values for comparison of dissolution data of tablet formulations were found to be less than 50 indicating that drug release profiles of the formulations in presence and absence of rat caecal content are dissimilar. So it was concluded that in
presence of rat caecal contents the drug release from the formulations was increased significantly due to bacterial enzymatic degradation of gums present in the rat caecal content.

The Thus further study was carried out by using simplex lattice design to study the synergistic effect of OG and HPMC on tablet properties.

Simplex lattice design

As per the mixture design the total amount of excipients was maintained for total weight of 400mg (OG, HPMC and MCC) and the Theophylline content was 200mg so that the total weight of tablet was adjusted to 600mg. Based on design, fourteen tablet formulations (SD01-SD14) were prepared as shown in Table 3.13. These prepared tablets were evaluated for weight variation, friability, hardness, drug content and drug release study for 5h (Rel₅h) as per the procedure described in methodology section 4.2.5. Results of prepared tablets for weight variation (≤0.84%), friability (≤0.87%) and hardness (3.5 ± 0.09kg/cm² – 4.5 ± 0.07kg/cm²) are presented in Table 4.27. Drug content was found to be in range of 98%-102% indicating uniformity in drug content as per Indian Pharmacopoeia.

Simplex lattice design was applied to study the influences of various compositions of OG and HPMC on drug release for 5h (Rel₅h). As per design, after producing and analyzing the formulations, response was applied to fit the appropriate model (linear or quadratic). The model was tested for goodness of fit ($R^2$) and analysis of variance (ANOVA) was applied to verify the adequacy of the regression model in terms of a lack-of-fit test (Table 4.28). As per the ANOVA report for Rel₅h, a quadratic design model appears that did not show significant lack of fit thus the used model was statistically accepted because of high F value (20.45) and low probability values (less than 0.0500). The "Lack of Fit F-value" of 41.90 implies the Lack of Fit is significant. In this case Linear Mixture Components, AB, AC are significant model terms (A=OG, B= HPMC, C= MCC). The statistical properties of model were diagnosed by
normal probability plot of residuals and data points were almost linear thus, there were no signs of any problems in our data (Figure 2.23). Thus, our data selected were good enough.

The below equation was generated from the data.

\[ \text{Rel}_{5h} = 35.06A + 30.11B + 23.90C + 66.72AB + 46.56AC + 12.84BC \] ------[14]

R-Square= 0.9191

Further the effect of variables on \( \text{Rel}_{5h} \) could be explored by superimposing different points through contour plot (Figure 2.24) and surface plot (Figure 2.25).

The results of dissolution study showed that the rate of drug release from these matrix tablets was mainly influenced by OG concentration. As the amount of the OG increases the extent of drug release decreases. This could be attributed to an increase in the density of the gum matrix and the diffusional path length that the drug has to traverse. Among prepared formulations (Table 3.13), duplicate formulations SD03 (\( \text{Rel}_{5h}=38.98\% \)) and SD12 (\( \text{Rel}_{5h}=38.44\% \)) containing higher amount of OG (300mg), relative amount of HPMC (100mg) and no MCC gave restricted drug release in upper GIT. These higher amounts of OG and presence of HPMC in matrix tablet prevents penetration of dissolution fluid in to tablet formulation. Because of relatively low microbial population in the upper GIT there might be less chance of degradation of OG in upper GIT which restricted the drug release and also because of partial swelling of HPMC that forms resistive layers around the tablet and prevent the drug release in upper GIT. A combination of the anionic okra gum with non ionic HPMC seems to produce a synergistic increase in viscosity. This may be attributed to the stronger hydrogen bonding between the okra gum and HPMC leading to stronger physical cross linking between the polymers. Interaction between non ionic and ionic polymers has been reported to be greater than between molecules of the same species (Traconis et al. 1997). These restricted
drug release in upper GIT would be suitable for colonic delivery of Theophylline and thus drug release study for these formulations (SD03 and SD12) was continued for sufficient time (next 3h) in phosphate buffer saline (pH 6.8) containing 4% rat caecal contents. Cumulative percentage drug release of prepared tablets (SD01-SD14) is presented in Table 4.29 and Table 4.30. Comparative dissolution profiles of prepared tablets (SD01-SD14) are shown in Figure 2.26. Formulations SD03 and SD12 showed fast drug release within 3h of dissolution in presence of rat caecal content. The results clearly demonstrate that OG is susceptible to enzymatic action of rat caecal contents as the percentage drug released in the presence of caecal matter is better than without caecal matter.

Remaining formulations (Table 3.13) showed relatively higher drug release in upper GIT. These might be due to lower concentration of OG in the formulations. The drug controlling nature of HPMC depends on viscosity which was found to be 157cps. Thus HPMC itself alone was not able to prevent the drug release for this much time and leads to drug release in upper GIT. MCC was found to act negatively on the hardness of tablets and gave tablets with poor hardness and thus tablets would no longer remain intact and releases the drug in upper GIT.

Preparation of matrix tablets by 5% starch paste

Tablet formulations OG24 to OG27 (Table 3.14) and OG28 to OG29 (Table 3.15) containing increasing concentrations of OG and combinations of OG with GG, respectively were prepared by using 5% starch paste as binder. These tablets were evaluated for weight variation, friability, hardness, drug content uniformity and Rel_{5h}. The results of weight variation (<1.4%), friability (<0.36%), hardness (3.4 ± 0.14kg/cm² to 4.5 ± 0.11kg/cm²) and drug content (96.31% to 102.3%) are shown in Table 4.31. Values of Rel_{5h} were found to be decreased with increasing concentration of OG and GG in the formulations (42.35% to 12.21%). Formulation (OG27) containing higher concentration of OG (400mg) showed restricted drug release in upper GIT (12.21%). This is due
to higher density of gum matrix at higher concentration in the formulation resulted in an increased diffusional path length that controls the drug release in upper GIT. At the end of the experiment the tablet retained its shape indicating that the drug release is by matrix diffusion controlled mechanism and not by erosion. The drug delivery systems targeted to the colon should not only protect the drug from being released in the physiological environment of stomach and small intestine, but also release the drug in colon after enzymatic degradation by colonic bacteria. Hence, drug release study was carried out with PBS pH 6.8 in presence or absence of rat caecal content. Cumulative percentage drug release from the formulations OG24-OG29 in presence and absence of rat caecal content were shown in Table 4.32, Table 4.33 and Table 4.34. Comparison of dissolution profiles for the various formulations OG24-OG29 is shown in Figure 2.27. Dissolution study in presence or absence of rat caecal content revealed that drug release in presence of rat caecal content was increased to a great extent as compared to drug release in absence of rat caecal content (control) for the above said formulations. Moreover, similarity factor ($f_2$) was calculated to compare dissolution profiles for the formulations (OG24-OG29) in presence and absence of rat caecal content. The $f_2$ values for comparison of dissolution data of tablet formulations were found to be less than 50 indicating that drug release profiles of the formulations in presence and absence of rat caecal content are dissimilar. So it was concluded that in presence of rat caecal contents the drug release from the various formulations was increased significantly.

Hence further study was performed based on the combination of okra gum and guar gum by employing factorial design.

Preparation of matrix tablets using Factorial design

In present section an attempt has been made to evaluate the synergistic effect of okra gum and guar gum in which the low swelling characteristics of okra gum in acidic pH and better tableting properties of guar gum were rationalized. Guar
gum has extensively used as carrier for effective colonic drug delivery system. It was found that the hydrophilic nature of guar gum leads to extensive swelling that might be responsible for premature release of drug in upper GIT. As a solution to this problem researcher have used many approaches viz. chemical modification of guar gum to reduce swelling characteristics, use of combination of guar gum with hydrophobic polymers as carrier, use of hydrophobic coating over the guar gum formulation etc. Thus, the combination of guar gum and okra gum might reduce the swelling of matrices that would minimize the drug release in upper GIT. To know the best combination, $3^2$ factorial design approach was used and according to which nine batches of tablets were prepared using independent variables ($X_1$, amount of okra gum and $X_2$, amount of guar gum) to check their effect on dependent parameters ($Rel_{5h}$ and $Rel_{8h}$) as shown in Table 3.16. The statistical analysis of the factorial batches was performed by multiple regression analysis using Microsoft Excel followed by ANOVA.

The tablets of all batches were evaluated for weight variation, friability, hardness and content uniformity. \textit{In vitro} dissolution study of these formulations was performed as per the procedure described in methodology section in the presence of simulated colonic fluids for 8h after completing the first 5h of dissolution study in 0.1N\textsubscript{HCl} (900mL) for 2h followed by phosphate buffer saline pH 7.4 (900mL) for 3h. The results of weight variation ($\leq 1.46\%$), friability ($\leq 0.64\%$), hardness ($4.25 \pm 0.22$kg/cm$^2$ to $5.01 \pm 0.11$kg/cm$^2$) and drug content ($97.80\%$ to $101.2\%$) are shown in Table 4.35. The drug delivery systems targeted to the colon should not only protect the drug from being released in the physiological environment of stomach and small intestine, but also release the drug in colon after enzymatic degradation by colonic bacteria. Hence, drug release study was carried out with PBS pH 6.8 in presence or absence of rat caecal content. Cumulative percentage drug release from the formulations (FS1-FS9) in presence and absence of rat caecal content are shown in Table 4.36. Comparison of dissolution profiles for the formulations (FS1-FS9) is shown in Figure 2.28.
To elucidate the influence of okra gum and guar gum on drug release, following polynomial equations were evolved for $\text{Rel}_{5h}$ and $\text{Rel}_{8h}$.

$\text{Rel}_{5h}=30.83 - 11.05X_1 - 3.35X_2 - 1.85X_1X_1 - 0.5X_2X_2 - 0.02X_1X_2$\[15\]

$R^2 = 0.987746$

$\text{Rel}_{8h}=86.08 - 11.05X_1 - 3.38X_2 - 5.91X_1X_1 - 1.22X_2X_2 - 1.29X_1X_2$\[16\]

$R^2 = 0.988177$

Concerning $\text{Rel}_{5h}$ and $\text{Rel}_{8h}$, the results of multiple linear regression analysis showed that both the coefficients $b_1$ and $b_2$ bear a negative sign. It was found that with increase in the concentration of OG and GG in the formulation the values of $\text{Rel}_{5h}$ and $\text{Rel}_{8h}$ decreased. It is possible that at higher gums concentration, Theophylline is trapped in smaller polymer cells and it is structured by its close proximity to the polymer molecules. So, increasing the amount of the gum in the formulations increased the time it took for the drug to leave the formulation and retard release of drug into the medium. The $\text{rel}_{5h}$ and $\text{rel}_{8h}$ for all the batches FS1 to FS9 varied from 13.24% to 43.09% and 62.88% to 92.55%, respectively (Table 4.37) with good correlation coefficient as 0.994 and 0.993, respectively. Equation 15 and 16 indicates that both the concentration of the $X_1$ and $X_2$ are responsible to decrease in the values of $\text{Rel}_{5h}$ and $\text{Rel}_{8h}$. Effect of $X_1$ and $X_2$ on $\text{Rel}_{5h}$ (Figure 2.29) and $\text{Rel}_{8h}$ (Figure 2.30) are shown by surface plots.

The dissolution profiles indicate that the drug release is proportional to the amount of okra gum and guar gum present in matrices. It was found that with increasing concentration of gums in the formulations drug release was decreased. Restricted drug release in upper GIT was found for the formulation containing higher amount of OG. At lower level of $X_1$ (OG) and $X_2$ (GG), premature drug release was observed. This may be due to guar gum present in the formulations. Guar gum swells in presence of aqueous medium and forms water permeable pores through which diffusion of drug occurs that responsible for premature release of drug. Drug release study for above said formulations in presence of rat caecal content showed higher drug release. This may be due
to anaerobic bacteria present in rat caecal content which might get acted upon by enzymatic action on matrix tablet containing natural polysaccharides (OG and GG) and leads to degradation of OG and GG with fast drug release.

To study the mechanism of drug release from the prepared matrices the release data were fitted to various mathematical models like First order, zero order, Hixoncrowell, peppas and matrix type using software PCP Disso V 3.0 (Paradkar et al., 2003; Anagha et al., 2010; Purushothaman et al., 2010). These mathematical models are useful when attempting to elucidate and understand the mechanism of drug release from matrix formulations and their use is well documented (Syed et al., 2009; Vijayalakshami et al., 2008; Ashmitha et al., 2008; Lutifi et al., 2008). When dissolution data are fitted to these empirical models, a high $R^2$ value indicates the appropriateness of the model to describing the possible mechanism of drug release. According to PCP DISSO V 3.0, the absorbencies at respective time intervals for all formulations were fed up in software with all details regarding conditions and in doing this software gave respective $R^2$ values for respective models. In present investigation it was found that all formulations FS1 and FS9 follow zero order release kinetics (Table 4.38). The release exponent $n$ is an indicative of the mechanics of drug release from the formulation. A value of 0.5 is indicative of diffusion-controlled drug release and 1.0 indicates swelling-controlled drug release. A value of $n$ between 0.5 and 1.0 indicates anomalous transport, i.e. both swelling- and diffusion-controlled release mechanisms. Matrix tablets have $n$ -values in the range of 0.45-0.89, signifying anomalous transport (Siepmam and Peppas, 2001; Ritger and Peppas, 1987). The value of $n$ for all the formulations (FS1-FS9) is presented in Table 4.38. The range of this $n$ value is 0.6431 to 0.7458 indicating anomalous transport. The value of $n$ was found to be decreased as the concentration of the polymer increased.
**In vivo pharmacokinetic study in rabbits**

Figure 2.31 showed peaks of blank plasma. It is clear from Figure 2.32 that good peak separation between Theophylline and caffeine (IS) under the applied HPLC conditions with reasonable retention times of 5.936 min and 8.620 min, respectively. No interference was observed, with blank rabbit plasma. Figure 2.33 shows standard calibration curve that was obtained by plotting the mean peak height ratios of Theophylline to caffeine (IS) against the corresponding Theophylline concentrations in the range from 0.5 μg/mL to 25 μg/mL (Table 4.39).

Formulations FS9, OG10 and OG27 containing 200mg Theophylline were chosen on the basis of in-vitro drug release study (restricted drug release in upper GIT and abrupt drug release on arriving colon) as dosage forms for administration. Theo-SR, a marketed formulation containing 200mg Theophylline was also selected as dosage form for administration for comparison of pharmacokinetic parameters. The tablet formulations containing drug were administered to rabbits with sufficient flush of water in fasting conditions which was assisted by a local veterinary doctor. Blood samples (1 mL) were collected from marginal ear vein at before dosing (zero hour) and at predetermined time intervals after dosing and quantitative determination of drug in plasma was performed by HPLC assay to generate plasma concentration time profiles for above said formulations.

The area under the curve (AUC$_{0-t}$) was estimated by the linear trapezoidal rule and AUC$_{0-\infty}$ was calculated by equation AUC$_{0-t}$ + $C_t$ / $k_e$, where $C_t$ is the last measurable concentration and $k_e$ the elimination rate constant which was determined from the slope of the linear portion of graph plotted between logarithm of plasma concentration and time. The peak plasma concentration ($C_{max}$, μg/mL) and corresponding time to peak ($T_{max}$, h) were determined by the inspection of the individual drug plasma concentration-time profiles. $T_{1/2}$ was determined using the equation $T_{1/2} = 0.693/K_e$. 

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The comparative plasma concentrations at different time intervals of prepared formulations (OG10, OG27 and FS9) with TheoSR (marketed formulation) are depicted in Table 4.40, Table 4.41 and Table 4.42, respectively. Plasma concentration profiles at different time intervals of prepared formulations (OG10, OG27 and FS9) with TheoSR (marketed formulation) are depicted in Figure 2.34, Figure 2.35 and Figure 2.36, respectively. The values of $C_{\text{max}}$ were found to be 20.12 ± 2.34μg/mL, 16.45 ±1.97μg/mL, 19.24 ±1.96μg/mL and 20.31 ±1.50μg/mL for the formulations TheoSR, OG10, OG27 and FS9, respectively. The values of $T_{\text{max}}$ were found to be 4.2h, 6h, 5.2h and 5.1h for TheoSR, OG10, OG27 and FS9, respectively. In vivo pharmacokinetic parameters for optimized formulations (OG10, OG27 and FS9) and marketed formulation (TheoSR) are summarized in Table 4.43. It was found that the absorption was relatively rapid with marketed formulation (TheoSR) as indicated by low $T_{\text{max}}$ value (4.2h); whereas the prepared colon targeted formulations exhibited delayed absorption as demonstrated by high $T_{\text{max}}$ values (6h, 5.2h and 5.1h for OG10, OG27 and FS9, respectively). This indicates that the absorption of Theophylline from the prepared formulations was most likely occurred in the lower GI tract of the rabbit. The delayed onset of Theophylline absorption can be attributed to the presence of the okra gum in the tablets, which has prevented the drug release in the upper GIT. The half-life of marketed formulation (TheoSR) was found to be 7.0h, which specifies the relatively rapid removal of drug from plasma and the rapid elimination of drug was further supported by relatively high elimination rate constant (0.099h$^{-1}$). On the contrary, the formulated colon targeted tablets exhibited high half-life (11.55h, 15.4h and 15.75h for OG10, OG27 and FS9, respectively) and low elimination rate constant values (0.06h$^{-1}$, 0.045h$^{-1}$ and 0.044h$^{-1}$ for OG10, OG27 and FS9, respectively) indicating that drug remains in the body for a long period of time and exhibits prolonged effect. Moreover, the Analysis of variance (ANOVA) for data of AUC$_{0-t}$ showed no statistically significant difference ($p<0.05$) between the prepared formulations (OG10, OG27 and FS9) when compared with marketed formulation (TheoSR). This suggests that the
prepared formulations have similar rate and extent of drug absorption. Based on these results it can be predicted that prepared colon specific formulations showed minimum drug absorption in upper GIT. This indicates the good control over drug release in upper GIT with abrupt drug release as tablet formulations reaches the lower part of GIT.

**Stability study**

In view of the potential utility of the formulations OG10, OG27 and FS9 for colonic release of Theophylline, stability study was carried out by storing the formulations at 40°C/75% RH for 6 months to access the long term stability. There was no change in the physical properties of these formulations at the end of storage period. The dissolution study was conducted for the said formulations in SGF, SIF and SCF as described in methodology section 4.2.5 after storage. No significant differences were observed in the cumulative percentage release of Theophylline from the formulations OG10 (Table 4.44), OG27 (Table 4.45) and FS9 (Table 4.46) stored at 40°C/75% RH for 6 months compared to the release of the same formulation before storage. The insignificant changes in the physical appearance, drug content and dissolution profile of these formulations after storage at 40°C/75% RH for 6 months indicate that the formulations could have a minimum shelf life of 2 years.
DICLOFENAC SODIUM

Identification of drug Identification of drug

Identification of Diclofenac sodium was performed by melting point study and IR spectroscopy study. Melting point of Diclofenac sodium was found in accordance with the standard melting point of Diclofenac sodium as per BP. Data of IR vibrations and its frequency were depicted in Table 4.47 and they were in accordance with standard IR spectrum of Diclofenac sodium (Figure 2.37).

Preformulation study by Differential Scanning Calorimetry

The DSC thermogram of the drug (Figure 2.38) depicts a sharp endothermic peak at 282.30°C corresponding to the melting transition temperature of Diclofenac sodium followed by an exotherm. This result is indicative of melting of drug followed by decomposition. The melting point of the drug was 284°C with an enthalpy variation (ΔH) of 19.446 Jg⁻¹ (within the 265–295°C range). The DSC thermogram (Figure 2.39) of core tablet of drug (C05) showed similar peaks corresponding to pure drug indicated the absence of well defined chemical interaction between the drug and excipients.

Spectro photometric analysis of Diclofenac sodium

Determination of λ_max of Diclofenac sodium in PBS pH 6.8

The UV visible spectrum of Diclofenac sodium in PBS pH 6.8 is shown in spectrum (Figure 2.40) and it is evident that the λ_max corresponds to 274nm.

Standard curve of Diclofenac sodium in different media

The absorbance values of different concentration of Diclofenac sodium (2µg/mL to 14µg/mL) in different media viz. distilled water, phosphate buffer saline pH 7.4 and phosphate buffer saline pH 6.8 are depicted in Table 4.48. The values of R² were found to be 0.998 (Figure 2.41), 0.970 (Figure 2.42) and 0.988 (Figure 2.43) for distilled water, phosphate buffer saline pH 7.4 and
phosphate buffer saline pH 6.8, respectively which indicate the accuracy of results.

**Formulation of fast disintegrating core tablets**

Fast disintegrating core tablets of Diclofenac sodium were prepared by using direct compression method that would allow the core tablets to disintegrate rapidly once the coat material is digested by the resident microflora of the colon. Tablet formulations (C01-C04) were prepared by using various disintegrating agents like chitosan, starch, pectin and lactose with magnesium stearate as lubricant for different tablet formulations (Table 3.17). The compression force was adjusted to give core tablets with hardness of 2kg/cm² to 3kg/cm². Prepared formulations were evaluated for weight variation, hardness, drug content and disintegration time (DT) using disintegration test apparatus. Results of weight variation (≤3.94%), hardness (2.1±0.09kg/cm² – 2.5±0.08kg/cm²), drug content (97.48% - 101.78%) and DT (38sec - 180sec) are depicted in Table 4.49. Further tablet formulations (C05-C13) were prepared by using MCC as diluents with various disintegrating agents like chitosan, starch, pectin and lactose. Magnesium stearate was used as lubricant for the above said tablet formulations (Table 3.18). The compression force was adjusted to prepare core tablets with hardness of 2kg/cm² to 3kg/cm². Prepared formulations were evaluated for weight variation, hardness, drug content and disintegration time (DT) using disintegration test apparatus. Results of weight variation (≤3.66%), hardness (2.1±0.34kg/cm² – 3.2±0.34kg/cm²), drug content (96.75% - 101.3%) and DT (18sec to MT 600sec) are depicted in Table 4.50. Average weight of the core tablets was fixed at the lowest possible level (155mg) to accommodate maximum amount of coat material over the core tablet and the average percentage deviation of core tablet was within the official limit (within 7.5%). and the core tablet formulation (C05) was disintegrated within 18sec showing required fast disintegration characteristics of core tablet. Diclofenac sodium core tablet formulation (C05) contains fast disintegrating additives like microcrystalline cellulose (50mg) and starch
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(50mg) which might have contributed for such a fast disintegration of core tablets (disintegration time=18sec) that would ensures abrupt and complete release of drug when the dosage form reaches colon. The fast disintegrating characteristics of the core tablet prevent it from being the rate limiting factor. Hence, core tablet formulation (C05) containing starch along with MCC was selected for further study.

Compression coating of fast disintegrating core tablets

Wet granulation method for the formulation of compression coat

Formulations CC14 to CC17 (Table 3.19) and CC18 to CC21 (Table 3.20) containing various concentration of OG was prepared by using 5% starch paste and 4% PVPK30 as binders, respectively. These tablets were evaluated for weight variation, friability, hardness and Rel$_{5h}$. The results of weight variation ($\leq 1.35\%$), friability ($\leq 0.89\%$) and hardness ($3.1 \pm 0.14\text{kg/cm}^2$ to $3.7 \pm 0.16\text{kg/cm}^2$) for the formulations (CC14-CC17) are depicted in Table 4.51. Values of Rel$_{5h}$ were found to be decreased with increasing concentration of OG in the formulations (25.34 to 11.14\%). Cumulative percentage drug release from the formulations (CC14-CC17) is shown in Table 4.52. Comparison of dissolution profiles for the formulations (CC14-CC17) is shown in Figure 2.44. The drug delivery systems targeted to the colon should not only protect the drug from being released in the physiological environment of stomach and small intestine, but also release the drug in colon after enzymatic degradation by colonic bacteria. Hence, drug release study was carried out with PBS pH 6.8 in presence or absence of rat caecal content for the formulation (CC17) because it shows restricted drug release in upper GIT (11.14\%). Cumulative percentage drug release in presence or absence of rat caecal content for the formulation (CC17) is tabulated in Table 4.53. Comparison of dissolution profiles for the formulation (CC17) in presence or absence of rat caecal content is shown in Figure 2.45. The percent drug released from tablets coated with coat formulation CC17 (500mg OG) was found to increase from 5h onwards indicating the commencement of breaking of gum coats in presence of rat...
Discussion

caecal content. The percent drug released after 8h of testing was 84.24% in presence of rat caecal content as compared to 35.24% in absence of rat caecal content. This difference in drug release was studied using similarity factor \( f_2 \). Dissolution profiles for the formulation (CC17) in presence and absence of rat caecal content was compared and \( f_2 \) value was found to be less than 50 indicating that drug release profiles of the formulation in presence and absence of rat caecal content are dissimilar. The study shows that the release of drug in the physiological environment of colon is due to microbial degradation of OG coat in presence of rat caecal content to ensure that the drug release was not due to mechanical erosion which is likely to occur because of bowel movement in the human.

The results of weight variation (\( \leq 1.42\% \)), friability (\( \leq 0.89\% \)) and hardness (3.1 ± 0.09kg/cm\(^2\) to 3.6 ± 0.11kg/cm\(^2\)) for the formulations (CC18-CC21) are depicted in Table 4.54. Values of Rel\(_{5h}\) were found to be decreased with increasing concentration of OG in the formulations (16.24% to 5.14%). This may be due to increased thickness of coat with increase OG concentration that act as resistance and thus retarded the drug release. Cumulative percentage drug release from the formulations (CC18-CC21) is shown in Table 4.55. Comparison of dissolution profiles for the formulations (CC18-CC21) is shown in Figure 2.46. The drug delivery systems targeted to the colon should not only protect the drug from being released in the physiological environment of stomach and small intestine, but also release the drug in colon after enzymatic degradation by colonic bacteria. Hence, drug release study was carried out with PBS pH 6.8 in presence or absence of rat caecal content for the formulation (CC21) because it shows restricted drug release in upper GIT (5.14%). Cumulative percentage drug release for the formulation (CC21) in presence or absence of rat caecal content is tabulated in Table 4.56. Comparison of dissolution profiles for the formulation (CC21) in presence or absence of rat caecal content is shown in Figure 2.47. The percent drug released from tablets coated with coat formulation CC21 (500mg OG) was found to increase from 5h onwards indicating the commencement of breaking of gum coats in presence of
rat caecal content. During drug release study, the percentage drug released after 8h was 80.14% in presence of rat caecal content as compared to 30.24% in absence of rat caecal content. This significant increase in drug release was explained by similarity factor ($f_2$). The $f_2$ value for dissolution data of tablet formulation (CC21) in presence and absence of rat caecal content was found to be less than 50 indicating that drug release profiles of the formulation (CC21) in presence and absence of rat caecal content are dissimilar. It may be due to bacterial population present in the rat caecal content that uses OG coat as a substrate and carrying out its hydrolysis with subsequent release of drug. It was found that PVPK30 as binder restricted the drug release in upper GIT (5.14%) and thus further batches were prepared using PVPK30.

To study synergistic action of OG and GG, compression coats were given using combinations of OG:GG viz. 25:75, 50:50, 75:25 to prepare core coat tablet formulations (CC22-CC24) as shown in Table 3.21. These formulations were evaluated for weight variation, friability, hardness and Rel$_{5h}$. The results of weight variation ($\leq 1.67\%$), friability ($\leq 0.89\%$) and hardness (3.1 ± 0.16kg/cm$^2$ to 3.7 ± 0.31kg/cm$^2$) for the formulations (CC22-CC24) are depicted in Table 4.57. Values of Rel$_{5h}$ were found to be decreased with increasing concentration of OG in the formulations (20.14% to 16.24%). Cumulative percentage drug release from the formulations (CC22-CC24) is shown in Table 4.58. Comparison of dissolution profiles for the formulations (CC22-CC24) is shown in Figure 2.48. It was found that with formulations (CC24) containing higher amount of OG could control the drug release in upper GIT (16.24%). This may be due to increased thickness of coat with increase OG concentration that act as resistance and thus retarded the drug release.

Direct compression method for the formulation of compression coat

A direct compression method was used to prepare coat which is to be given on core tablet with the aim of controlling further drug release in upper GIT. Tablet formulations (CC25-CC28) containing increasing concentration of OG were prepared where coat was given by direct compression method (Table 3.22).
These prepared tablets were evaluated for weight variation, friability, hardness and Rel₅₉. The results of weight variation (≤1.99%), friability (≤0.89%) and hardness (3.1 ± 0.14kg/cm² to 3.7 ± 0.09kg/cm²) for the formulations (CC25-CC28) are depicted in Table 4.59. Values of Rel₅₉ were found to be decreased with increasing concentration of OG in the formulations (11.14% to 3.11%). This was attributed to the increased thickness with increase OG concentration that act as resistance and thus retarded the drug release. Cumulative percentage drug release from the formulations (CC25-CC28) is shown in Table 4.60. Comparison of dissolution profiles for the formulations (CC25-CC28) is shown in Figure 2.49. The drug delivery systems targeted to the colon should not only protect the drug from being released in the physiological environment of stomach and small intestine, but also release the drug in colon after enzymatic degradation by colonic bacteria. Hence, drug release study was carried out with PBS pH 6.8 in presence or absence of rat caecal content. Drug release study in presence or absence of rat caecal content was performed for the formulation (CC28) that restricted drug release in upper GIT. Cumulative percentage drug release for the formulation (CC28) in presence or absence of rat caecal content is tabulated in Table 4.61. Comparison of dissolution profiles for the formulation (CC28) in presence or absence of rat caecal content is shown in Figure 2.50. The percent drug released from tablets coated with coat formulation CC28 (500mg OG) was found to increase from 5h onwards indicating the commencement of breaking of gum coats. The percent drug released after 8h was 78.24% in presence of rat caecal content as compared to 27.27% in absence of rat caecal content. Moreover, similarity factor \(f_2\) was used to compare dissolution profiles for the formulation (CC28) in presence and absence of rat caecal content. The \(f_2\) value for comparison of dissolution data of tablet formulations was found to be less than 50 indicating that drug release profiles of the formulation in presence and absence of rat caecal content are dissimilar. So it was concluded that in presence of rat caecal contents the drug release from the formulation was increased significantly.
Further batches of tablets (CC29-CC40) were prepared using combinations of OG and other polymers to check their synergistic effect on tablet properties. Various employed polymers with okra gum were HPMC, pectin, ethyl cellulose and guar gum (Table 3.23). These tablets were evaluated for weight variation, friability, hardness and Rel$_{5h}$. The weight variation ($\leq$1.9%), friability ($\leq$0.87%) and hardness (4.1 $\pm$ 0.14kg/cm$^2$ to 4.7 $\pm$ 0.57kg/cm$^2$) for the formulations (CC29-CC40) are depicted in Table 4.62. Values of Rel$_{5h}$ were found to be decreased with increasing concentration of OG in the formulations (36.45% to 11.64%). During dissolution study some tablet formulations containing combinations of OG with HPMC (CC29) and EC (CC35-CC37) were broken down in two halves and thus their percentage drug release was not mentioned and these combinations were not used in further study. Formulations (CC32-CC34) containing combination of OG and pectin were found to release the drug relatively faster in upper GIT and hence not included for further study. Formulations (CC38-CC40) containing combination of OG and GG were effective to retard the drug release in upper GIT and gave faster drug release when it reaches to the colon due to synergistic effect of OG (low water solubility) and GG (tableting properties).

Based on this preliminary study it was found that directly compressed formulations containing combinations of OG:GG as coat were able to control the drug release in upper GIT to a great extent and hence further study was employed using direct compression method.

Factorial design

A factorial design was employed to find out the best combination of OG and GG that controls further drug release in upper GIT and upon arrival to colon that releases the drug as quickly as possible.

Tablet formulations (FC1-FC9) were prepared using $3^2$ factorial design wherein $X_1$, ratio of OG to GG (25:75, 50:50, 75:25) and $X_2$, total weight of coat (250mg, 350mg, 450mg) were selected as independent variables (Table 3.24)
and their effect on $Rel_{5h}$ was studied. These tablets were evaluated for weight variation, friability, hardness and $Rel_{5h}$. The results of weight variation ($\leq 1.91\%$), friability ($\leq 0.77\%$) and hardness ($4.23 \pm 0.08\text{kg/cm}^2$ to $5.14 \pm 0.14\text{kg/cm}^2$) for the formulations (FC1-FC9) are depicted in Table 4.63. Values of $Rel_{5h}$ were found to be decreased with increasing concentration of OG in the formulations (32.12% to 5.45%). The data clearly indicates that the dependent variables are strongly dependent on the independent variables.

The polynomial equation can be used to draw a conclusion after considering the magnitude of coefficient and the mathematical sign it carries (positive or negative). In order to determine the levels of factors which yield optimum dissolution responses, mathematical relationships were generated between the dependent and independent variables using the analysis tool pack (Microsoft Excel).

The equations of the response $Rel_{5h}$ is given below:

$$Rel_{5h} = 18.57 - 5.518X_1 - 7.7066X_2 - 2.705X_1X_1 + 0.53X_2X_2 + 1.6725X_1X_2$$

R square= 0.9543

Percentage drug released for five hour ($Rel_{5h}$) for the 9 batches showed wide variation in the response ranging from a minimum 5.45% to a maximum of 32.12%. This large difference between the minimum and maximum value indicates that the ratio of OG to GG and coating thickness of the formulation (total weight of coat) plays an important role in controlling drug release in upper GIT. Figure 2.51 shows the surface plot for $Rel_{5h}$.

Cumulative percentage drug release from the formulations (FC1-FC9) is shown in Table 4.64 and Table 4.65. Comparison of dissolution profiles for the formulations (FC1-FC9) is shown in Figure 2.52. Among these batches, formulation (FC9) can be considered as optimized formulation because it gives minimum drug release in upper GIT (5.45%). The drug delivery systems targeted to the colon should not only protect the drug from being released in the
physiological environment of stomach and small intestine, but also release the drug in colon after enzymatic degradation by colonic bacteria. Hence, drug release study was carried out in PBS pH 6.8 in presence or absence of rat caecal content. Drug release study in presence or absence of rat caecal content was performed for the formulation (FC9) that restricted drug release in upper GIT. The percent drug released from tablets coated with coat formulation FC9 ($X_1=75:25$ and $X_2=450\text{mg}$) was found to increase from 5h onwards indicating the commencement of breaking of gum coats in presence of rat caecal content. The percentage drug released after 8h was 97.64% in presence of rat caecal content as compared to 35.24% in absence of rat caecal content. On coming into contact with biological fluids the gum mixture swells up and the drug release takes place by diffusion. Mechanical erosion of swollen gums layer follows. Unless the swollen gums layer erodes, further hydration and swelling of gum coat does not takes place. On reaching colonic environment, the swollen gums layer would be acted upon by bacterial enzymes and release the drug contained in the swollen gums layer. This was confirmed by similarity factor ($f_2$) which is used to compare dissolution profiles. The $f_2$ value of dissolution data of tablet formulation (FC9) was found to be less than 50 indicating that drug release profiles of the formulations in presence and absence of rat caecal content are dissimilar. So it was concluded that in presence of rat caecal contents the drug release from the formulation was increased significantly. Cumulative percentage drug release for the formulation (FC9) is tabulated in Table 4.66. Comparison of dissolution profiles for the formulation (FC9) is shown in Figure 2.53.

Drug release kinetics

To study the mechanism of drug release from the prepared matrices the release data were fitted to various mathematical models like First order, zero order, Hixoncrowell, peppas and matrix type using software PCP Disso V 3.0 (Paradkar et al., 2003; Anagha et al., 2010; Purushothaman et al., 2010). These mathematical models are useful when attempting to elucidate and understand
the mechanism of drug release from matrix formulations and their use is well documented (Syed et al., 2009; Vijayalakshami et al., 2008; Ashmitha et al., 2008; Lutifi et al., 2008). When dissolution data are fitted to these empirical models, a high $R^2$ value indicates the appropriateness of the model to describing the possible mechanism of drug release. According to PCP DISSO V 3.0, the absorbencies at respective time intervals for the formulation (FC9) were fed up in software with all details regarding conditions and in doing this software gave respective $R^2$ values for respective models. In present investigation best fit model was found to be Korsmeyer-Peppas ($R^2=0.9983$) for the formulation FC9 (Table 4.67). The release exponent $n$ is an indicative of the mechanics of drug release from the formulation. A value of 0.5 is indicative of diffusion-controlled drug release and 1.0 indicates swelling-controlled drug release. A value of $n$ between 0.5 and 1.0 indicates anomalous transport, i.e. both swelling- and diffusion-controlled release mechanisms. Matrix tablets have $n$ -values in the range of 0.45-0.89, signifying anomalous transport (Siepmam and Peppas, 2001; Ritger and Peppas, 1987). The $n$ value was found to be 0.756 indicating anomalous transport. In this formulation, ratio of OG to GG ($X_1$) decreases the hydration of matrix and retards the release owing to its relative hydrophobic property where as total weight of coat ($X_2$) retards the release by diffusion mechanism.

**Scanning electron microscopy (SEM)**

During dissolution study, formulation (FC9) shows controlled drug release in upper GIT with subsequent rapid drug release in colonic fluid was subjected to scanning electron microscopy (SEM) to check the surface integrity of tablet during different phases of dissolution. Images were taken at different time intervals viz. 0h, 2h, 5h and 6h and shown in Figure 2.54. These time intervals were selected because that reflects the change of dissolution medium viz. 0h for image for fresh tablet, 2h in 0.1N HCl (pH 1.2), 5h in PBS (pH 7.4) and 6h in PBS (pH 6.8) containing rat caecal content. SEM image of fresh tablet formulation (FC9) at 0h showed smooth surface. SEM image of same tablet at
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2h exhibited less porosity, after 5h showed increased porosity. The low porosity in 0.1N HCl (pH 1.2) may be due low solubility of OG in 0.1N HCl (pH 1.2). The higher porosity of tablet surface in PBS (pH 7.4) may be due to its solubility at near neutral pH (pH 6.5) (Kalu et al., 2007). SEM image of same tablet after 6h in PBS containing rat caecal content showed larger pore formation. The microbiota of rat caecal content might act upon the natural polysaccharides and stimulates microbial degradation that resulted in the larger pore size. The increased porosity of the surface promoted the increase of drug diffusion and the opening of the channels with abrupt release of drug. These pictures clearly show that pore formation is the most important mechanism of drug release.

In vivo pharmacokinetic study

It is clear from Figure 2.55 that good peak separation between Diclofenac sodium and naproxen (IS) under the applied HPLC conditions with reasonable retention times of 7.52min and 2.30min, respectively. A standard curve was constructed using a fixed amount of naproxen (µg/mL) as an internal standard and varying the amount of Diclofenac sodium (Table 4.68). The curve was linear over the range of 0.5µg/mL to 30µg/mL \( (R^2=0.987) \), the value of intercept was 0.087 (Figure 2.56). The pharmacokinetic parameters were calculated from the plasma level data obtained from the individual rabbit and presented as mean ± SD. A plot of the mean plasma concentration versus time has been constructed for each of the three treatments.

Formulations CC28 and FC9 containing 50mg Diclofenac sodium were chosen on the basis of in-vitro drug release study (restricted drug release in upper GIT and abrupt drug release on arriving colon) as dosage forms for administration. Voveran, a marketed conventional formulation containing 50mg Diclofenac sodium was also selected as dosage form for administration for comparison. The tablet formulations containing drug were administered to rabbits with sufficient flush of water in fasting conditions which was assisted by a local veterinary doctor. Blood samples (1mL) were collected from marginal ear vein
at before dosing (zero hour) and at predetermined time intervals after dosing and quantitative determination of drug in plasma was performed by HPLC assay to generate plasma concentration time profiles for above said formulations.

The area under the curve (AUC\textsubscript{0\,-\,t}) was estimated by the linear trapezoidal rule and AUC\textsubscript{0\,-\,\infty} was calculated by equation AUC\textsubscript{0\,-\,t} + C\textsubscript{t} / k\textscript{e}, where C\textsubscript{t} is the last measurable concentration and k\textsubscript{e} the elimination rate constant which was determined from the slope of the linear portion of graph plotted between logarithm of plasma concentration and time. The peak plasma concentration (C\textsubscript{max}, μg/mL) and corresponding time to peak (T\textsubscript{max}, h) were determined by the inspection of the individual drug plasma concentration-time profiles. T\textsubscript{1/2} was determined using the equation T\textsubscript{1/2} = 0.693/K\textsubscript{e}.

The comparative plasma concentrations at different time intervals of prepared formulations (CC28 and FC9) with Voveran (marketed formulation) are depicted in Table 4.69 and Table 4.70, respectively. Plasma concentration profiles at different time intervals of prepared formulations (CC28 and FC9) with Voveran (marketed formulation) are shown in Figure 2.57 and Figure 2.58, respectively. The values of C\textsubscript{max} were found to be 23.64 ± 2.11μg/mL, 21.21 ± 2.24μg/mL and 22.31±2.64μg/mL for the formulations Voveran (marketed formulation), CC28 and FC9, respectively. In vivo pharmacokinetic parameters for optimized formulations (CC28 and FC9) and marketed formulation (Voveran) are summarized in Table 4.43. It was found that the absorption was relatively rapid with marketed formulation (Voveran) as indicated by low T\textsubscript{max} value (1.9h); whereas the prepared colon targeted formulations exhibited delayed absorption as demonstrated by high T\textsubscript{max} values (6.2h and 6.0h for CC28 and FC9, respectively). This indicates that the absorption of Diclofenac sodium from the prepared formulations was most likely occurred in the lower GI tract of the rabbit. The delayed onset of Diclofenac sodium absorption can be attributed to the presence of the okra gum coating over the core tablet, which has prevented the drug release in the upper GIT. The half-life of marketed formulation (Voveran) was found to be 3.01h,
which specifies the relatively rapid removal of drug from plasma and the rapid elimination of drug was further supported by relatively high elimination rate constant (0.23h\(^{-1}\)). On the contrary, the prepared colon targeted formulations exhibited high half-life viz. 6.3h and 8.15h for CC28 and FC9, respectively and low elimination rate constant values viz. 0.11h\(^{-1}\) and 0.085h\(^{-1}\) for CC28 and FC9 indicating that drug remains in the body for a longer period of time and exhibits the prolonged effect. Moreover, the Analysis of variance (ANOVA) for data of AUC\(_{0-1}\) showed no statistically significant difference (\(p<0.05\)) between the prepared formulations (CC28 and FC9) when compared with marketed formulation (Voveran). Based on these results it can be predicted that prepared colon specific formulations (CC28 and FC9) showed a lag time of 4h before finally showing maximum concentration (\(C_{\text{max}}\)). This indicates the good control over drug release in upper GIT with abrupt drug release as the tablet formulations reaches the lower part of GIT.

**Stability study**

In view of the potential utility of the formulations CC28 and FC9 for colonic release of Diclofenac sodium, stability studies were carried out by storing the formulation at 40\(^0\)C/75% RH for 6 months to access the long term stability (Krishnaiah et al., 2002). There was no change in the physical properties of these formulations at the end of storage period. When the dissolution study was conducted in SGF, SIF and colonic fluid as described in methodology section 4.2.5. No significant differences (\(f_2\) values were ranged from 50 to 100 for all the formulations) were observed in the cumulative percentage of Diclofenac sodium released from the formulations CC28 (Table 4.72) and FC9 (Table 4.73) stored at 40\(^0\)C/75% RH for 6 months when compared to that released from same formulation before storage. The insignificant changes were observed in the physical appearance, drug content and dissolution profile of these formulations after storage at 40\(^0\)C/75% RH for 6 months which indicate the formulation could have a minimum shelf life of 2 years.